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STARCH

Chemistry and Technology

SECOND EDITION

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CHAPTER I

HISTORY AND FUTURE EXPECTATION OF STARCH USE

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I. Introduction

Although the past is usually prologue to the future, industrial usage of starch will be greater than normal methods of economic projection would indicate. Even though starch usage has shown a steady, rapid rate of growth over an extended period of time, new events are increasing demand for starch in an ever upward direction.

The birth of enzyme engineering made possible low cost conversion of starch to D-glucose and on to an equilibrium mixture of D-glucose and D-fructose equivalent in sweetness to invert sugar from cane or beet. This process alone made possible an immediate seizure of 30% of the sucrose market in the United States and doubled the amount of starch produced by the wet milling industry. With development of even more sophisticated methods of enzyme chemistry, it will become possible to transform starch into novel molecules possessing new properties suitable for entirely new applications.

A second event that makes projections for starch use difficult, but still suggests an upward demand, is the increased price of energy. This places new usage requirements on low cost starch to serve as a source of alcohol, components for plastics, special absorbents, paper extenders, oil well drilling muds, and as an additive of tertiary oil recovery systems, the potential requirement for the latter being enormous.

A third, large area of starch usage, more accurately predictable, is as a basic

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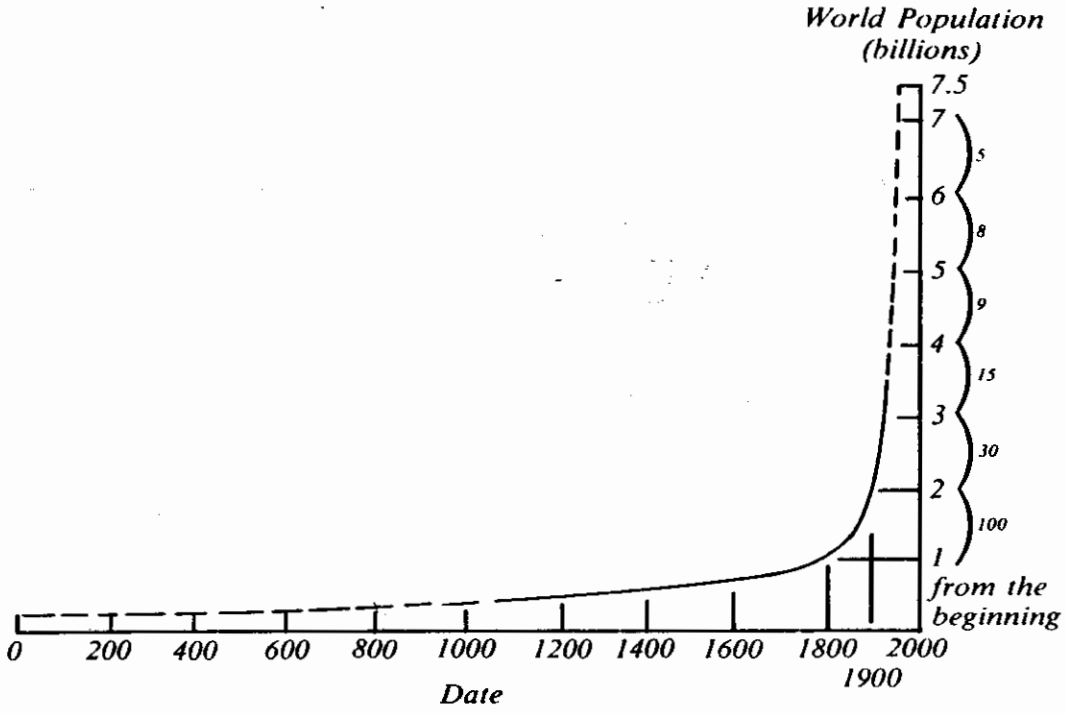

FIG. 1.—World population growth.

food to supply nutritional requirements of the growing world population (Fig. 1). Traditionally, carbohydrates supply 80% of the calories consumed by the human population with two-thirds of these calories coming from starch. Now that starch can be economically converted to sweeteners, it will supply a greater proportion of nutritional calories. Then too, as meat costs rise, the amount of carbohydrates consumed in the diet of populations in developed countries may increase over present levels.

Each of these considerations indicates a firm place for starch as a future industrial raw material.

II. EARLY HISTORY

Starchy foods have always been an item in the diet of man. It is natural, therefore, that the practical use of starch products, and later of starch itself, should have developed in an early period. Some developments are cloaked in the predawn twilight of the unrecorded past. Strips of Egyptian papyrus, cemented together with a starchy adhesive and dated to the predynastic period of 3500–4000 B.C., give evidence of the early use of starch. However, a description of starch and its application is not found until much more recent times. The historian and philosopher, Caius Plinius Secundus (Pliny the Elder, 23–74 A.D.), described documents of 130 B.C. made by sizing papyrus with modified wheat starch to produce a smooth surface. The adhesive was made from fine ground

wheat flour, boiled with a dilute solution of vinegar. The paste was spread over strips of papyrus; the strips beaten with a mallet and further strips lapped over the edges to give a broader sheet. Pliny stated that the 200-year-old sheets which he observed were still in good condition. Pliny also described the use of starch to whiten cloth and to powder hair. Chinese paper documents of about 312 A.D. are reported to contain starch size (*1*). Later, Chinese documents were first coated with a high-fluidity starch to provide resistance to ink penetration and then covered with powdered starch to provide weight and thickness. Starches from rice, wheat, and barley were commonly used at that time.

A procedure for starch production was given in some detail in a Roman treatise by Cato in about 184 B.C. (*2*). Grain was steeped in water for 10 days, pressed and mixed with fresh water, then filtered on a linen cloth, after which the starch in the filtrate was allowed to settle, washed with water, and finally dried in the sun.

Modified starches used for adhesives or to provide a sweet molasses developed at an early period. Hydrolysis was a common method of modification, and vinegar as well as amylolytic enzymes were used. Abu Mansur (*3*), an Arabian teacher and pharmacologist, was acquainted with the use of saliva on starch for producing an "artificial honey" used for treating wounds.

Starch was introduced into Northern Europe to stiffen linen, possibly early in the fourteenth century. Colored and uncolored starches were used as cosmetics. Uncolored starch was used principally as a hair powder. Blue starch was employed by the Puritans until its use was banned by Queen Elizabeth in 1596. Yellow starch was quite fashionable until a notorious woman prisoner wearing a bright yellow-starched ruffle was publicly executed. Red starch cosmetics remained in fashion for many years.

Leeuwenhoek (*4*), the inventor of the microscope, made intensive and accurate observations of starch granules in 1719 and a dictionary (*5*) describing starch and its manufacture was published in 1744.

As starch became a more important industrial commodity, work was done on its modification. This included the great discovery of Kirchoff in 1811 that sugar could be produced from potato starch by acid-catalyzed hydrolysis. Then, there was the accidental discovery of the torrefaction method for producing dextrins, now termed British gums. In 1821, a fire occurred in a Dublin textile mill that utilized starch as a size. After the blaze was extinguished, one of the workmen noticed that some of the starch had been turned brown by the heat and dissolved easily in water to produce a thick, adhesive paste. The roasting of new starch was repeated and the product was shown to have the useful properties previously observed. In this way, heat dextrinization became known and subsequently elaborated into wide usage.

In Europe, early use of wheat and barley starch gave way to white potato starch, which was produced in large quantities in The Netherlands and Germany.

III. American Development

The first American wheat starch plant was started by Gilbert (*6*) at Utica, New York in 1807, but was converted to one producing corn starch in 1849. The change from wheat to corn starch began with Thomas Kingsford's development in 1842 of a manufacturing process in which crude corn starch was purified by alkaline treatment. The wheat starch plant of George Fox started in 1824 at Cincinnati was also converted to a corn starch plant in 1854. The William Colgate wheat starch plant built in Jersey City in 1827 was changed to a corn starch plant in 1844. Thomas Kingsford was hired by the Colgate Company in 1832 and became its superintendent in 1842. Another early wheat starch plant was that of T. Barnett built in Philadelphia in 1817 before moving to Knowlton, Pennsylvania, in 1879, and ceasing operation in 1895. In that year, there were five wheat starch and sixteen corn starch plants operating in the United States. The corn starch plants produced 200 million pounds of starch per year.

Potato starch manufacture began in 1820 in Hillsborough County, New Hampshire. Usage of potato starch grew rapidly until in 1895, sixty-four factories were in operation, of which fourty-four were in Maine. They produced 24 million pounds of starch per year during approximately 3 months of operation. Most of the starch was sold to the textile industry.

Rice starch manufacture, using caustic treatment of rice, began in 1815. However, production did not increase significantly and the little rice starch later used was mainly imported.

Following Kirchoff's finding (*7*) in 1811 that sweet dextrose (D-glucose) could be produced by acid-catalyzed hydrolysis of starch, factories were built to produce sweet syrups. Within a year, factories were built in Munich, Dresden, Bochman, and Thorin. By 1876, Germany alone had forty-seven dextrose syrup factories using potato starch to produce 33 million pounds of syrup and 11 million pounds of solid sweetener.

An American syrup plant of 30 gallon per day capacity was started in 1831 at Sacket Harbor, New York, but soon failed. A large plant was built by the Hamlins in 1873 at Buffalo, New York. In 1883, the Chicago Sugar Refinery Company began the manufacture of dextrose but soon added starch and modified starches.

In 1880, there were one-hundred forty starch plants producing corn, wheat, potato, and rice starches. Most were small and located along the Atlantic coast. The largest was the Glen Cove, New York, plant producing 65 million pounds of corn starch per year.

Thomas Kingsford and Son built a starch plant at Oswego, New York, in 1849 that continued to operate until destroyed by fire in 1903. The corn starch plant built in 1855 by Wright Duryea of Glen Cove, Long Island, became the largest starch factory in the United States in 1891, grinding 7000 bushels of corn per

day. First designated the Duryea Starch Works, the name was changed in 1881 to the Glen Cove Manufacturing Company.

The corn starch plant built in Indianapolis, Indiana, in 1867 by William F. Piel, Sr., was destroyed by fire in 1872. A second plant was closed by the city because of the environmental problem of fermentation odor from gluten overflow water. This led to the construction of a larger and more efficient plant in 1873.

By 1890 the number of American starch plants had decreased to eighty, producing 240 million pounds of starch per year. Although many small plants were built, they could not compete, and in 1890 a consolidation of several occurred to become the National Starch Manufacturing Company of Kentucky, representing 70% of the corn starch capacity. The consolidation included the Duryea plant at Glen Cove, the Piel plant at Indianapolis, and eighteen plants in Ohio, Illinois, Iowa, and Indiana. The firm built a new modern plant at Des Moines, Iowa, in 1899. Some of the independents then combined in the same year to form the United Starch Company. Among these independents were Thomas Kingsford and Sons, the U.S. Sugar Refining Company at Waukegan, Illinois, founded in 1888, the Argo Starch Company at Nebraska City, Nebraska, founded in 1894, and the Sioux City Starch Company, founded in 1896. In 1900 the United Starch Company and the National Starch Manufacturing Company combined to form the National Starch Company of New Jersey. Kingsford reported (*6*) that in 1895 there were sixteen corn starch factories producing 200 million pounds of starch per year and grinding 29,000 bushels of corn per day.

In 1902, the Glucose Sugar Refining Company joined with the National Starch Company to form the Corn Products Company that then represented 80% of the corn starch industry with a daily grind of 65,000 bushels. The Corn Products Company also included the Illinois Sugar Refining Company at Pekin, Illinois, and 49% of the stock of the New York Glucose Company formed by E. T. Bedford and Associates at Edgewater, New Jersey. A disastrous price war then ensued between Corn Products Company and the New York Glucose Company because of the refusal of the latter to allow Corn Products Company more than 50% stock ownership. The result was a merger in 1906 of the two combatants to form the Corn Products Refining Company, with a grinding capacity of 140,000 bushels of corn per day, which soon was reduced to 110,000 bushels or 74% of the United States total.

Also, in 1906 the Western Glucose Company was incorporated to become later the American Maize Company, the National Candy Company was incorporated to become later the Clinton Sugar Refining Company, and the Staley Company of Decatur, Illinois, began. In 1913 antitrust action caused the Corn Products Refining Company to spin off the Granite City, Illinois, the Davenport, Iowa, and the Oswego plants.

In 1958 Corn Products Refining Company acquired Best Foods Company and changed the parent name once again to Corn Products Company.

Anheuser-Busch Companies, Inc., which built a cornstarch plant in 1923 at St. Louis, Missouri, sold its only starch-producing facility at Lafayette, Indiana, to the A. E. Staley Manufacturing Company in 1981.

1. Present American Companies

Today, CPC International Inc. (Corn Products Company) has plants at Argo, Illinois; Pekin, Illinois; North Kansas City, Missouri; Stockton, California; and Winston-Salem, North Carolina.

The A. E. Staley Manufacturing Company that built its Decatur, Illinois plant in 1912 now has plants at Decatur, Illinois; Morrisville, Pennsylvania; Lafayette, Indiana; and Loudon, Tennessee.

American Maize-Products Company, started at Roby, Indiana, in 1908, now has plants at Hammond, Indiana and Decatur, Alabama.

Penick and Ford Ltd., beginning in 1902 as the Douglas Starch Company at Cedar Rapids, Iowa, is now a subsidiary of Univar Corporation.

Clinton Corn Processing Company, Inc., with plants at Clinton, Iowa, and Montezuma, New York, formerly a subsidiary of Nabisco Brands Incorporated, has been leased to ADM Foods.

National Starch and Chemical Corporation, with a plant at Indianapolis, Indiana, is a subsidiary of Unilever Corporation.

The Hubinger Company, with a plant at Keokuk, Iowa, is a subsidiary of H. J. Heinz Company.

ADM Foods, a division of Archer Daniels Midland Company, with plants at Cedar Rapids, Iowa, and Decatur, Illinois, has leased Clinton Corn Processing Company, Inc.

Amstar Corporation has a plant at Dimmitt, Texas.

Cargill, Incorporated has plants at Cedar Rapids, Iowa; Dayton, Ohio; and Memphis, Tennessee.

IV. Waxy Corn

Starch has many applications in the food and non-food industry, but usual starches are mixtures of linear and branched polysaccharides. These molecules have very different physical properties, and starches composed of only one component have special properties that open new and broader applications and lead to specialized uses for which regular starches are unsuited.

One unusual genetic variety of corn arose in China among the corn plants transferred from the Americas. This was a corn whose starch granules contained no linear molecules, but only branched molecules. It stained red with iodine, not blue as do ordinary starches. When the corn kernel was cut with a knife, the cut surface appeared shiny as though it contained wax. Hence the corn was called

waxy corn, though no wax was present. Waxy corn was brought to the United States in the first years of the twentieth century and remained a curiosity at experiment stations until World War II cut off the supply of tapioca from the East Indies. During a search for a replacement, waxy corn starch was found suitable. Iowa state geneticists developed the waxy corn in their possessions to a high-yielding hybrid. It was introduced as a contract crop and has continued to serve as a valuable speciality starch. It has continued in use because of its unique properties and because the corn crop also supplies quality oil and protein. Although other similar starches, such as waxy sorghum and glutinous rice, are composed only of branched molecules, they have not had the industrial acceptance of waxy corn.

V. High-Amylose Corn

The linear polysaccharide of normal starches has the common property of linear molecules in its ability to form junction zones, crystallize in part, and form films and even fibers of high strength and flexibility. Amylose can simulate the behavior of cellulose in many important applications. Thus, in 1946, R. L. Whistler and H. H. Kramer, a geneticist, set about to produce a corn modification that would be the opposite of waxy corn, in which the starch would be composed only of linear (amylose) molecules. Much was learned about the effect of specialized genes on endosperm composition. The two investigators were able to raise the amylose content from the normal of 25% to 65%. Toward the end of their work, and subsequently, others entered into genetic programs; the amylose content was increased to 85%, with 65–70% being common in varieties made commercially.

As world demands for polymers grow and prices of polymers made from petrochemical sources increase, it may be expected that starch amylose, and likely high-amylose corn, will assume a stronger place as an industrial commodity.

VI. Future of Starch

Changing world economics is making it more practical to obtain chemicals from agriculture. Both academic and industrial investigators are giving more attention to developing technologies for converting agricultural products to chemicals and to methods for modifying starch, cellulose, and sucrose.

Starch is the lowest priced and most abundant worldwide commodity. It is produced in most countries and is available at low cost in all countries. Its level price over many years is impressive and makes it especially attractive as an industrial raw material (Table I). Its production by the wet-milling industry has continued to increase (Table II) and may increase at a faster rate as starch takes

Table I

Corn Price

Year	*Dollars per pound*
1972	0.021
1973	0.037
1974	0.053
1975	0.048
1976	0.045
1977	0.037
1978	0.042
1979	0.048
1980	0.051
1981	0.043

Table II

Wet-Milled Corn

Year	*Bushels* $\times$ 10^6	*Pounds* $\times$ 10^9
1972	265	14.8
1976	350	19.6
1977	365	21.0
1978	400	22.4
1979	430	24.1
1980	480	26.9 (projected)

Table III

U.S. Population Sweetener Consumption (Pounds per Capita)

Year	*Glucose syrup*	*Glucose–fructose syrup*	*Sucrose*
1960	9.4	0	97.4
1965	12.3	0	96.8
1975	16.2	6.8	91.5
1978	17.6	13.9	91.5
1980	18.2	15.5	91.3 (projected)

more of the sweetener market (Table III) and as governments subsidize ethanol production.

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EXHIBIT 7
A126 – A131

[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH BRANCH[1]]

The Structure of a New Starch of High Amylose Content[2]

BY I. A. WOLFF, B. T. HOFREITER, P. R. WATSON, W. L. DEATHERAGE AND M. M. MACMASTERS

RECEIVED SEPTEMBER 13, 1954

Structural studies are reported on a starch containing about 50% amylose, derived from a corn variety having a high starch content. Like wrinkled-seeded peas, this corn contains a starch which is not only high in amylose but has an amylopectin fraction of unique structure and properties, intermediate between usual amylose and amylopectin fractions. Both inner and outer branches of the new amylopectin are longer than those of normal corn amylopectin. The new starch was fractionated after pretreatment in liquid ammonia. Its fractions were characterized by their extent of conversion by β-amylase, reducing power, iodine-complexing capacity, viscosity and periodate oxidation analysis. New modifications of the latter technique are reported which provide more suitable controls for the oxidation and furnish an estimate of the proportion of reducing end groups of a polysaccharide.

The potential utility of the linear starch component (amylose) or its chemical derivatives for the preparation of formed items such as films, fibers and molded articles[3] has motivated a continuing search for improved procedures for the fractionation of ordinary starches as well as for new plant sources with a starch of high amylose content.

While some progress has been made toward the development of better fractionation methods,[4] their commercial use has not been undertaken.

The starches from wrinkled-seed peas[5] and from certain varieties of sweet corn[6] have been reported to have high amylose contents varying from about 60 up to as much as 98%. Other workers have failed to confirm the latter value.[7] In any case, the total starch content of these materials is low. The most encouraging genetic development has been the discovery of a corn variety having a starch high in amylose content and which, unlike the previously studied "high-amylose corn" varieties, has a high starch content.[7b] A more detailed examination of the structure of starch from this new hybrid by study of its fractions appeared desirable and forms the basis of this report.

The high gelatinization temperature of this starch gave rise to difficulty in effecting its complete dispersion even when the usual conditions of starch fractionation[8] were modified to include drastic mechanical disintegration of the swollen granules. Solution of the starch in alkali, followed by neutralization, enabled satisfactory separation of the fractions by butanol precipitation. This procedure, however, necessitated repeated reprecipitation of the amylopectin fraction to obtain a product of low ash content.[9] The preferred procedure of fractionation was to pregelatinize the starch in liquid ammonia,[10] which converted it to a form more easily dissolved in hot water saturated with *n*-butyl alcohol. This is probably a phenomenon involving reduction in crystallinity similar to that effected by the action of various amines on cellulose.[11] Higher crystallinity in this native starch is apparently distributed through the entire granules since even when its granules were fractured by grinding they appeared microscopically to be disrupted by water to a lesser extent than damaged granules of the usual types of corn starch.

The starch fractions, after separation and (in case of the amylose) purification, were characterized by extent of conversion by β-amylase, reducing power, iodine sorption both potentiometrically and spectrophotometrically, viscosity and periodate oxidation analysis. The amylopectin fraction was further characterized by fractional precipitation with alcohol and by optical rotation of its tricarbanilate derivative.

Experimental

Raw Materials.—The starch was isolated from corn produced in the 1952 crop year by the Bear Hybrid Corn Company, Inc., and referred to by that company under the trade name of Amylomaize.[12] This starch is identical with that described by Deatherage, *et al.*[7b] The starch isolation was accomplished by conventional laboratory procedures[13] except that the sulfur dioxide content of the steep was progressively increased over a 48-hour period from 0.05 to 0.40% to simulate commercial countercurrent steeping procedures. The starch on a dry basis had 0.15% N, 0.035% P and 1.81% methanol-extractable material. This relatively high content of methanol extractables may be due to presence of some alcohol-soluble protein. Alternatively, there may be some relationship of the large amount of methanol-extractable material to the higher amylose content of the starch. Ability of amylose to complex with fatty material is well known. The starch was defatted by extraction with 85% methanol for fractionation by procedures B and C (see below).

The starch was incompletely dissolved by 0.1–0.2 *N* potassium hydroxide solution but was dispersed in 0.3–1.0 *N* potassium hydroxide. The rate and extent of solution

(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) Presented in two parts: Before the Division of Carbohydrate Chemistry at the 126th National Meeting of the American Chemical Society, New York, N. Y., September, 1954; and at the Midwest Regional Meeting of the American Chemical Society, Omaha, Nebr., November, 1954.

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(12) The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

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were not visibly different at room temperature from at refrigerator temperature (approximately 4–5°).

Fractionation of Amylomaize Starch. Procedure A.—Complete dispersion of the Amylomaize starch granules was not effected when a 3% suspension in water saturated with *n*-butyl alcohol was subjected, alternately, to periods of autoclaving at 15 p.s.i.g. and agitation of the hot suspension in a Waring blendor. Conventional procedures of dispersion in water–butanol mixture without pretreatment of the starch were therefore abandoned.

Procedure B.—The procedure of Potter, *et al.*,[9] was followed, except that *n*-butyl alcohol instead of amyl alcohol was used as the complexing agent. The amylopectin solution was first separated from the amylose–butanol complex at room temperature and then refrigerated as recommended by those authors. Separation of a small quantity of precipitate occurred, which was indistinguishable by potentiometric iodine titration from the major portion of the amylopectin. Over-all total solids recovery in this procedure was 92%. The yield of amylopectin fraction was 41%. The yield of amylose was not calculated. Amylose samples taken at successive stages of purification had iodine affinities of 169, 190, 191 and 196 mg./g.

Procedure C.—The starch (100 g., dry basis), which had been pretreated in liquid ammonia,[10] was slurried in 300 ml. of *n*-butyl alcohol. This slurry was added to a stirred mixture of 2700 ml. of water and 300 ml. of butanol at 90°. After a heating period of 1 hour, an additional 200 ml. of butanol was added and the mixture (*p*H 6.7) was autoclaved for 1 hour at 15 p.s.i.g., then allowed to cool slowly. The amylose was recrystallized from hot water saturated with butanol. The solutions were autoclaved for 1 hour at each recrystallization stage. The thrice-recrystallized amylose (43% yield) sorbed 200 mg. of iodine/g. The amylopectin fraction showed no inflection in its potentiometric iodine titration curve (see below).

Normal Starch Fractions.—Starch was fractionated according to the procedure of Schoch,[8] and the amylose fraction was recrystallized twice from hot butanol-saturated water. The respective iodine sorptions of the fraction were 193 and 12 mg. I_2/g.

Characterization of the Fractions. Extent of Conversion by β-Amylase.—β-Amylase was prepared from wheat flour by the method of Ballou and Luck[14] with an acid treatment of the aqueous enzyme-containing extract (0.5 hour at *p*H 3 and 0°) included for inactivation of a major portion of any α-amylase contamination.[15] Potency was determined by the method of Kneen and Sandstedt[16] except that the Somogyi procedure[17] was used for determination of reducing sugar. Activity is expressed as the grams of starch converted by 1 ml. of the β-amylase solution in 1 hour at 30°.

Amylopectins were converted to limit dextrins by allowing 11 or 23 units enzyme/g. substrate to act on a 4% solution for 48 hours at 30° in a solution preserved with toluene and buffered by *p*H 4.6–4.8. Under these conditions an amylopectin fraction from ordinary corn starch was converted to the extent of 56%, the amylopectin from Amylomaize starch was converted to the extent of 58% of the reducing sugar, estimated as maltose,[17] theoretically obtainable by complete hydrolysis of the fraction to that sugar.

High extents of conversion of amylose to maltose occurred only when high ratios of enzyme to substrate were used. The procedure of Bernfeld and Gürtler[18] was used except that the alkaline amylose solution was added all at once to the buffered enzyme. Extents of conversion of 0.08% amylose solutions at 30° in the presence of toluene and sodium acetate–acetic acid buffer were

Amylose sample	β-Amylase units/g. amylose	Conversion to maltose, % 2.5 hr.	24 hr.
Normal corn	874	82	94
Amylomaize	874	76	90
Normal corn	262	79	83
Amylomaize	262	75	79

No significant differences in convertibility were found between Amylomaize fractions obtained by the various fractionation procedures.

Reducing Power Determinations.—Reducing power determinations were carried out by the use of alkaline 3,5-dinitrosalicylic acid according to the procedure of Lansky, *et al.*,[19] and expressed in terms of degree of polymerization (DP). Values obtained were

	Normal corn starch	Amylomaize starch Procedure B	Procedure C
Amylose	320	320	285
Amylopectin	450	220	150

Potentiometric Iodine Sorption.—The fractions were analyzed for iodine affinity by the procedure of Bates, French and Rundle[20] as modified by Wilson, Schoch and Hudson.[21] The amylopectin fraction from Amylomaize starch showed no inflection in the titration curve. However, there is definite sorption of iodine, as indicated by the displacement of the curve from that of the blank (Fig. 1). Admixture of the amylopectin with a weighed amount of normal corn amylose of known sorption, to superimpose the inflection of the latter on the amylopectin curve, indicates the amylopectin fraction to sorb 50.5 mg. of iodine/g., which, if due to amylose, corresponds to an apparent amylose content of approximately 25%. The amylose fraction of Amylomaize starch, iodine affinities of which have already been cited, reacted in the usual fashion in the potentiometric iodine titration. The original, defatted Amylomaize starch sorbed 109 mg. of iodine/g. starch.

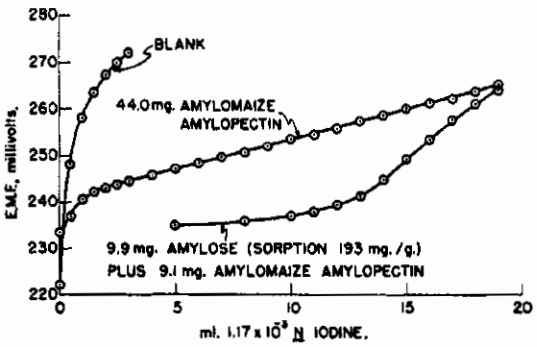

Fig. 1.—Potentiometric iodine titration curves of amylomaize amylopectin alone or in admixture with amylose.

Determination of Blue Values.—Blue values were determined by the procedure of McCready and Hassid[22] with the modifications that (a) samples were allowed to stand in 0.5 *N* sodium hydroxide overnight, then heated for 20 minutes at 60–63° under nitrogen for final dispersion; (b) final measurements were at 660 mμ and are reported as the optical density of a 1-cm. depth of solution; and (c) standards used for comparison were a recrystallized amylose fraction which sorbed 198 mg. of iodine/g., measured potentiometrically and the blank made with iodine–potassium iodide solution but containing no polysaccharide. For practical purpose of calculation, the blue value of the highly branched polysaccharide, corn glycogen, can be considered as zero (Fig. 2). The blue values (0.335–0.346) of the recrystallized amylose from Amylomaize starch were consistent with their purities as deduced from potentiometric iodine titration. The amylopectin fraction from Amylomaize starch had an apparent amylose content of 23–27% (blue values 0.078–0.092). A synthetic mixture of normal amylopectin (88%) and amylose (12%) having approximately the same blue value was used for comparative purposes. Spectral distribution curves of several of the solutions used for blue value determination are shown in Fig. 2. The Amylomaize starch had an apparent amylose content of 58%.

(14) G. A. Ballou and J. M. Luck, *J. Biol. Chem.*, **139**, 233 (1941).
(15) W. J. Olson, B. A. Burkhart and A. D. Dickson, *Cereal Chem.*, **20**, 126 (1943); E. Kneen, R. M. Sandstedt and C. M. Hollenbeck, *ibid.*, **20**, 399 (1943).
(16) E. Kneen and R. M. Sandstedt, *ibid.*, **18**, 237 (1941).
(17) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).
(18) P. Bernfeld and P. Gürtler, *Helv. Chim. Acta*, **31**, 106 (1948).
(19) S. Lansky, M. Kool and T. J. Schoch, THIS JOURNAL, **71**, 4066 (1949).
(20) F. L. Bates, D. French and R. E. Rundle, *ibid.*, **65**, 142 (1943).
(21) E. J. Wilson, Jr., T. J. Schoch and C. S. Hudson, *ibid.*, **65**, 1380 (1943).
(22) R. M. McCready and W. Z. Hassid, *ibid.*, **65**, 1154 (1943).

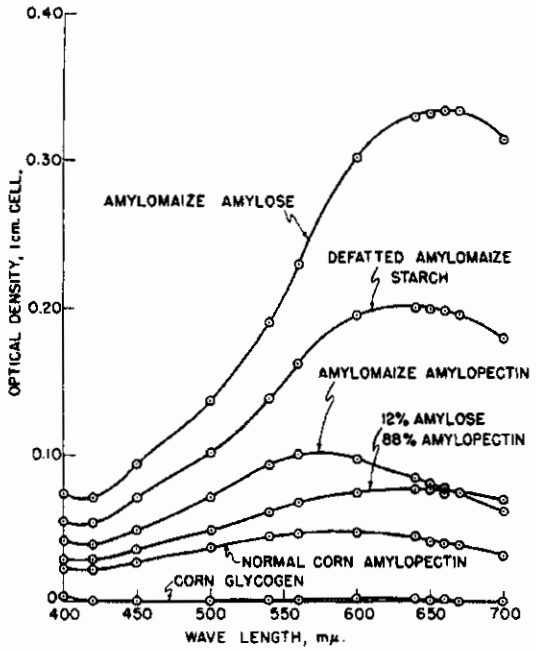

Fig. 2.—Spectral absorption of the polysaccharide–iodine complexes.

Viscosity Measurement.—Intrinsic viscosities were measured in 1 *N* potassium hydroxide by a procedure previously developed at this Laboratory.[23] The equations reported for calculation of intrinsic viscosity from measurements made at one concentration were found to be valid for amylose and amylopectin fractions of Amylomaize starch. Intrinsic viscosities of the fractions at 25° were: recrystallized amylose, 1.16–1.30; amylopectin, 1.12–1.29. There was as much variation in viscosity between different preparations following a given fractionation procedure as there was when different procedures were used. The fractions had viscosities of the same order of magnitude as fractions from ordinary corn starch.

Periodate Oxidations.—The average chain length (average number of anhydroglucose units per terminal non-reducing end group) of the various starch fractions was assessed by oxidation with sodium metaperiodate in the cold. Since amylaceous substrates have a tendency to over-oxidize, even at refrigerator temperatures, it has become customary to use a disaccharide as a reference standard. Although Potter, *et al.*,[9,24] found that maltose serves as a suitable standard and yields 3 moles of formic acid per mole sugar, other workers[25,26] have not had success with this procedure. The present authors, too, have found that maltose gives less than 3 moles of formic acid under the usual experimental conditions. Since the production of formic esters in periodate oxidation of appropriate sugars is now well substantiated,[27,28] it seemed reasonable to assume neither complete hydrolysis nor complete retention[29] of formic ester in oxidized maltose but to measure both the amount of free formic acid and that bound as formic ester. The procedure used for maltose was as follows: to 50 ml. of a precooled solution of 0.2220 g. of maltose hydrate in water was added 10.0 ml. of a solution of sodium metaperiodate containing 8 g./100 ml. Oxidations were carried out in the dark at 4–5°. At suitable time intervals, aliquots were withdrawn and 1 ml. of purified (distilled from KOH) ethylene glycol was added to react with the unused periodate. After 1 hour in the dark at room temperature, free formic acid was determined by titration with 0.01 *N* sodium hydroxide to the brom cresol purple end-point. After reaching the end-point, additional 0.01 *N* sodium hydroxide was added (approximately 1 meq. per meq. reducing end group), and this solution allowed to stand for 0.5 hour at room temperature to hydrolyze the formyl ester (up to 2 hours saponification time did not change the results). The solution was brought to the acid side with a known amount of 0.01 *N* sulfuric acid and again titrated to the brom cresol purple end-point with the alkali. The additional consumption of alkali, corrected for appropriate blanks and for the acid added, enabled calculation of the unhydrolyzed formyl ester. Results of the oxidation of maltose by this technique are shown in Fig. 3. The end-point of the reaction, when maltose is used as a reference standard, is taken at a total formic acid production of 3 moles/mole maltose. The authors have not verified that the acid titrated is exclusively formic. It is possible that acidic materials may be produced from the trialdehyde by the added alkali,[30] but this probably occurs to a negligible extent under the conditions used because of the rapid rate of hydrolysis of formate esters[28] and resultant consumption of the added alkali.

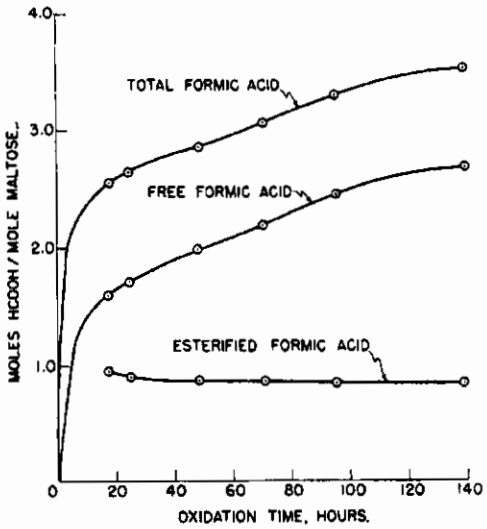

Fig. 3.—Production of formic acid by periodate oxidation of maltose.

The amylopectin samples (0.4750 g.) were oxidized and titrated in the same fashion as was the maltose, and were found to give appreciable amounts of titratable acid (Fig. 4) released by saponification. This is interpreted as being due to formyl ester at the reducing end group of the molecules. The reducing end group yields a total of 2 moles of formic acid. The average chain length may be considered as the reciprocal of the percentage of terminal non-reducing groups, or the reciprocal of the quantity [total HCOOH (moles/AGU) − 2 × ester formyl (moles/AGU)]. Calculated in this fashion (ester formyl corrected for percentage hydrolyzed, as indicated below), the average chain lengths of the normal corn starch amylopectin and the Amylomaize starch amylopectin were, respectively, 27 and 36. Furthermore, since 1 mole of formyl ester per molecule is produced, it should be possible to calculate from this figure an approximate number average molecular weight if no degradation occurred during the oxidation. Since the formyl ester of oxidized maltose is 13% hydrolyzed under our conditions at the end-point of the reaction, the assumption is made that a similar proportion of the ester at the amylopectin reducing end group is hydrolyzed. For normal corn amylopectin, for example, at 65 hours total formic acid (0.0413 mole/

(23) I. A. Wolff, L. J. Gundrum and C. E. Rist, THIS JOURNAL, **72**, 5188 (1950).

(24) A. L. Potter and W. Z. Hassid, *ibid.*, **70**, 3488 (1948).

(25) M. Morrison, A. C. Kuyper and J. M. Orten, *ibid.*, **75**, 1502 (1953).

(26) D. J. Manners, *Biochem. J.*, **55**, xx (1953).

(27) R. W. Lemieux and H. F. Bauer, *Can. J. Chem.*, **31**, 814 (1953).

(28) G. Neumüller and E. Vasseur, *Arkiv Kemi*, **5**, 235 (1953); F. S. H. Head and G. Hughes, *J. Chem. Soc.*, 603 (1954).

(29) K. H. Meyer and P. Rathgeb, *Helv. Chim. Acta*, **32**, 1102 (1949).

(30) F. S. H. Head, *J. Textile Inst.*, **38**, T389 (1947); E. M. Fry, E. J. Wilson, Jr., and C. S. Hudson, THIS JOURNAL, **64**, 872 (1942).

AGU) − free formic acid (0.0395 mole/AGU) = ester formyl (0.0018 mole/AGU) which corrected for 13% hydrolysis would be 0.0021 mole/AGU. This corresponds to a number average degree of polymerization of 476; the Amylomaize amylopectin, by similar calculation, had a DP_N of 386.

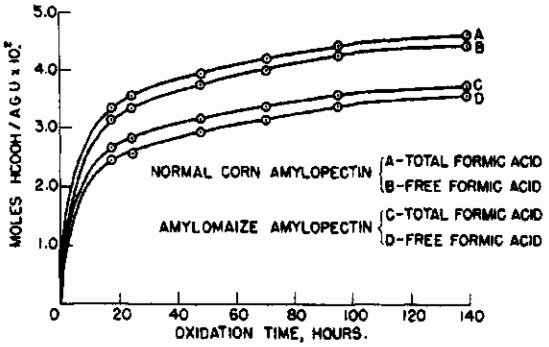

Fig. 4.—Production of formic acid by periodate oxidation of amylopectins.

Periodate oxidation of the amylose samples was carried out as before, but in the presence of 3% sodium chloride and a small amount of petroleum ether.[34] The tendency of the samples to clump and the smaller formic acid production from amylose make the results subject to somewhat greater error than in the case of amylopectins. The average chain lengths (calculated on the assumption that the molecules are linear)[34] for both normal corn amylose and Amylomaize amylose were 460 (total HCOOH acid production 0.0065 mole/AGU at 65 hours reaction time). The amount of ester formyl (corrected for 13% hydrolysis) at the end point was approximately one-fourth (0.0015 mole/AGU) of the total formic acid. This would indicate that on the average these amylose samples had about 2 (calcd. 2.3) chains per molecule. Kerr and Cleveland[31] have found corn amylose to be unbranched while Potter and Hassid[32] concluded that their corn amylose had on the average 2.9 chains per molecule.

Fractional Precipitation of Amylopectin with Methanol.—As a further comparison between Amylomaize amylopectin and normal corn amylopectin, 2% aqueous solutions of each were sub-fractionated with graded amounts of methanol at 25 ± 0.1°. The precipitated sub-fractions were sedimented by centrifugation and, after removal of the supernatant liquid, dissolved in water to a known volume and their amounts estimated by measurement of the optical rotation. The $[\alpha]^{25}D$ in water of both amylopectins was found to be +200 ± 5°. The original solutions were too turbid for more accurate measurement. Additional methanol was added to the supernatants and the process repeated. The data are shown graphically in Fig. 5.

Amylomaize amylopectin is more difficultly soluble in water than is normal corn amylopectin. It was necessary to autoclave the partial solution, prepared on a steam-bath, for 1 hour at 15 p.s.i.g. pressure before all of the swollen particles had dissolved. The autoclaved solution was more turbid than one similarly prepared from normal corn amylopectin. Furthermore, the Amylomaize amylopectin partially retrograded from solution on being refrigerated. A white flocculent precipitate, which was easily redissolved on warming, settled on standing. Certain of the sub-fractions also showed this type of retrogradation. Thus about two-thirds of the sub-fraction soluble in 50% methanol retrograded from aqueous solution in the cold.

Amylomaize Amylopectin Tricarbanilate.—Amylomaize amylopectin was converted to its tricarbanilate derivative by a procedure previously described.[33] *Anal.* Calcd. for $C_{77}H_{23}N_3O_8$: N, 8.09. Found: N, 8.03. This derivative was $[\alpha]^{25}D$ −76° (pyridine, *c* 1), a value intermediate between that of the normal corn starch fraction tricarbanilates, but falling closer to that of the amylose derivative.

(31) R. W. Kerr and F. C. Cleveland, THIS JOURNAL, **74**, 4046 (1952).

(32) A. L. Potter and W. Z. Hassid, *ibid.*, **70**, 8774 (1948).

(33) I. A. Wolff and C. E. Rist, *ibid.*, **70**, 2779 (1948).

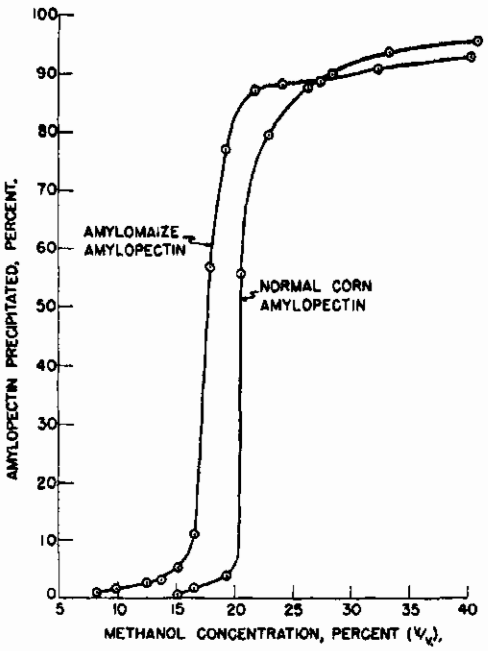

Fig. 5.—Fractional precipitation of amylopectins with methanol.

Discussion

The amylopectin fractions of starches having high amylose contents have been reported to be more branched[34] (for sweet corn) and less branched[9] (for wrinkled-seeded peas) than the corresponding fraction isolated from the usual types of starches. The amylose fractions were not found to differ from that of the normal starch from the plant source under study.

The starch from Amylomaize corn appears structurally to be very similar to if not identical with wrinkled-seeded pea starch.[9] Its amylose fraction is like that of normal corn starch in structure and molecular size. Thus the iodine sorption measured potentiometrically, blue value, intrinsic viscosity, formic acid production upon periodate oxidation and reducing power of Amylomaize amylose were identical, within limits of experimental error, with the values found for normal corn amylose. A new modification of the periodate oxidation technique showed a similarity in the number of chains per molecule. Since the conversion of an amylose fraction by β-amylase is dependent in such large measure on the completeness of its dispersion, no significance is attached at this time to the differences in rate of conversion (or extent at the end of 24 hours) found between the normal and Amylomaize amyloses.

In contrast, there is definite structural difference between amylopectin from normal corn and that from Amylomaize corn. The latter is more difficultly soluble in water and retrogrades rapidly from aqueous solutions in the cold. The viscosities of the amylopectin fractions are similar to each other and to those of the amylose fractions. Yet

(34) W. Dvonch, H. H. Kramer and R. L. Whistler, *Cereal Chem.*, **28**, 270 (1951).

the number average molecular weight of Amylomaize amylopectin as determined by alkaline dinitrosalicylic acid is less than that of amylose while that of normal corn amylopectin is greater. While these molecular weights may have no absolute significance,[19,35] taken as relative measures they enable the conclusion that Amylomaize amylopectin has a lower ratio of number average degree of polymerization to viscosity than does normal corn amylopectin. Two possible reasons for this might be (a) greater linearity of Amylomaize amylopectin, or (b) the existence of a type of molecular polydispersity for that fraction involving a greater proportion of quite high and quite low molecular weight components as compared with normal corn amylopectin. There is some evidence in favor of each of these possibilities, and it is quite probable that both factors are important. The iodine sorption, blue value, extent of conversion of β-amylase, periodate oxidation data, tendency toward retrogradation greater difficulty of solubility in water and rotation of the tricarbanilate derivative indicate that Amylomaize amylopectin has a greater degree of linearity than normal corn amylopectin. The precipitation curve (Fig. 5) of Amylomaize amylopectin has a slight discontinuity at a methanol concentration near 30% which may indicate some heterogeneity in that sample. Furthermore, there were portions which were precipitated at both lower and higher methanol concentration than corresponding normal corn amylopectin fractions. This would suggest the presence in the former of sub-fractions differing from one another by a greater amount in molecular structure, size or both. In a private communication Dr. T. J. Schoch has pointed out that methanol may be considered as a weak amylose-complexing agent and that the greater tendency of the Amylomaize amylopectin to precipitate with methanol may indicate greater linearity of structure.

A continuous variability in the lengths of linear portions of Amylomaize amylopectin would account[20] for the non-appearance of an inflection point in the iodine potentiometric titration curve, a possibility also considered by Potter, *et al.*[9]

Although polydisperse in size, and perhaps not homogeneous in chemical structure, Amylomaize amylopectin is best considered as a polysaccharide entity. Three lines of evidence indicate that Amylomaize amylopectin is not a mechanical mixture of amylose and amylopectin, but that it is a representative of a new polysaccharide type, intermediate in structure between amylose and amylopectin: (A) A 1% aqueous solution of Amylomaize amylopectin retrogrades to the extent of 94% on refrigeration, more than would be expected from admixture of a branched polysaccharide with 23–27% amylose even if the latter on retrogradation mechanically occluded some amylopectin. (B) In mechanical mixtures of amylose and amylopectin the former can be identified by an inflection in the potentiometric iodine titration curve. (C) The spectral distribution curve (Fig. 3) of the polysaccharide–iodine complex was quite different for Amylomaize amylopectin from that for a synthetic mixture of normal corn starch fractions having substantially the same blue value. The curves fall close to one another at 660 mμ, the wave length taken for calculation of apparent amylose, but the Amylomaize amylopectin–iodine complex showed an absorption maximum displaced toward the shorter wave lengths.

The existence of such intermediate fractions has been anticipated.[19,36,37]

Extension of this line of thought has important implications on our method of expression of the amylose content of starches such as Amylomaize. Rigorous assay of the true amylose content necessitates fractionation to establish the iodine-complexing capacity of the constituent fractions of a starch for use as standards. In the case of the 1952 crop year Amylomaize starch, use of the fractions originating from that starch as standards would (on the basis of blue value) indicate its amylose content as 47% instead of an apparent 53% if ordinary corn amylopectin was used as a standard or apparent 58% with an iodine blank as standard. If one wishes to report apparent amylose on the basis of blue values for surveying a series of starch samples, the use of a glycogen or an iodine blank as one standard (Fig. 2) is recommended. The potentiometric titration as conventionally used is, of course, a measure of apparent amylose content. Total iodine-sorptive capacity of a starch, even though not a measure of separable amylose fraction, may be found to correlate well with other starch properties such as film-forming ability, which are dependent on the total linear material present

If it is accepted that Amylomaize amylopectin is an intermediate type of amylaceous polysaccharide with an average chain length of 36 anhydroglucose units, comparisons are in order between the lengths of the inner and outer branches of this substance and those of normal corn amylopectin. Average chain length multiplied by extent of conversion by β-amylase plus 2[38] gives a figure approximating the outer-branch length. Inner-branch length may be taken as the difference between the average chain length and the outer-branch length, using the terminology and structural conceptions of other recent workers in the field.[38] The results of such calculations are

	Branch length	
	Outer	Inner
Normal corn amylopectin	17	10
Amylomaize amylopectin	23	13

It is seen that the difference in the amylopectins is reflected in both the inner- and outer-branch lengths.

Further structural studies on Amylomaize amylopectin through its sub-fractionation and by examination of its β-amylase limit dextrin are contemplated. Study of the fractions of other samples of starch of high amylose content derived from corn

(35) It is interesting to note that the DPn obtained by the new modification of the periodate oxidation procedure is of the same order of magnitude as that from the dinitrosalicylic acid procedure.

(36) R. W. Kerr and O. R. Trubell, *Paper Trade J.*, **117**, No. 15, 25 (1943).

(37) J. E. Hodge, E. M. Montgomery and G. E. Hilbert, *Cereal Chem.*, **25**, 19 (1948).

(38) B. Illingworth, J. Larner and G. T. Cori, *J. Biol. Chem.*, **199**, 631 (1952); J. Larner, B. Illingworth, G. T. Cori and C. F. Cori, *ibid.*, **199**, 641 (1952).

hybrids similar to the one presently considered should clarify the relationship, if any, between genetic composition of the corn and the type of amylopectin component present in the starch.

Acknowledgment.—The authors gratefully acknowledge the coöperation of R. P. Bear in furnishing the corn from which the starch was isolated. Thanks are also due to Mrs. Margaret M. Holzapfel for extracting the starch from the corn; R. L. Lohmar, Jr., for furnishing the corn glycogen; T. A. McGuire for determination of methanol extractables in the starch; Mrs. Phyllis L. Patrick for viscosity analyses and β-amylase convertibility determinations; J. W. Sloan for microscopic observations on the fractured starch granules; C. H. Van Etten for nitrogen and phosphorus analyses; B. H. Alexander for assistance in several periodate oxidation analyses; and C. E. Rist for advice and encouragement in the course of this work.

PEORIA, ILLINOIS

[CONTRIBUTION FROM THE STARCH AND DEXTROSE SECTION, NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Kinetics of Hydrolysis of Isomaltotriose and Isomaltotriitol[2]

BY R. W. JONES, R. J. DIMLER AND C. E. RIST

RECEIVED OCTOBER 21, 1953

Conflicting evidence appears in the literature regarding the relation between rate of acid hydrolysis of glucosidic bonds and their position in polysaccharide molecules. The present studies were undertaken to provide information on such a relationship in the α-1,6′-glucosidically linked homologous series of oligo- and polysaccharides. Attention has been centered on the first two members of this series, isomaltose and isomaltotriose, and the alcohols obtained by reduction of these sugars. Procedures employing quantitative paper chromatography were developed for the study of the kinetics of hydrolysis of these carbohydrates. In the series isomaltose, isomaltotriose and dextran, the over-all rate constant for hydrolysis decreases with increase of chain length, the rate constant for dextran B-512 being about one-third that for isomaltose. For the individual bonds in the reduced oligosaccharide, isomaltotriitol, the bond farthest removed from the sorbitol end is cleaved twice as fast as the other linkage. It is postulated, on the basis of these data and certain assumptions concerning the effects of reduction of isomaltotriose, that the non-reducing end bond in isomaltotriose is hydrolyzed about 1.7 times as fast as the reducing end bond.

Considerable success has been attained in recent calculations of length of external branches in dextran molecules on the basis of measurements of the amounts of glucose and low molecular weight oligosaccharides formed during partial acid hydrolysis of the dextrans.[3] The interpretation of the data has been dependent in part upon the knowledge of whether the position of an α-1,6′-glucosidic linkage in the polymer chain influences its rate of hydrolysis. Since there is a lack of agreement in the literature concerning the effect of the position of a bond on its hydrolysis rate constant, K, the present studies were undertaken to provide evidence of such effects in the α-1,6′-linked glucose polymers.

A greater rate of hydrolysis of terminal compared with internal bonds in polysaccharides has been postulated by Freudenberg, Carlqvist, and others on the basis of studies of acid hydrolysis of cellulose,[4-7] starch,[4a,5,8-10] glycogen,[11] Schardinger dextrins[12] and low-molecular weight oligosaccharides.[4b,7] This hypothesis was based on the increase in K during hydrolysis of polysaccharides and the extent to which the velocity of hydrolysis increased in the order cellulose, cellohexaose, cellopentaose, cellotetraose, cellotriose, cellobiose. Swanson and Cori,[13] however, failed to detect an increase in K during hydrolysis of polysaccharides from starch. Likewise they found no difference in hydrolysis rate constant for amylose and maltose. The results obtained by Swanson and Cori may reflect the effects of differences in hydrolysis conditions[11] or in analytical methods used.

In the present studies attention was centered on the first two members of the α-1,6′-glucosidically linked, homologous series, isomaltose and isomaltotriose, which had been made available as a result of their preparation by carbon column chromatography of enzymic hydrolyzates of dextran from *Leuconostoc mesenteroides* NRRL B-512.[14] A procedure employing quantitative paper chromatography was developed for determining the effect of chain length on the over-all rates of hydrolysis (total bond cleavage) of these oligosaccharides. In addition, chromatographic techniques facilitated measurement of the rate constants for hydrolysis of the individual bonds in the reduced trisaccharide, isomaltotriitol. These data provided a basis for estimating the rates of cleavage of the individual bonds in the parent trisaccharide, isomaltotriose, although certain assumptions were necessary to allow for the difference between the over-all hy-

(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) Presented before the Division of Carbohydrate Chemistry at the 124th National Meeting of the American Chemical Society, Chicago, Ill., September, 1953.

(3) R. W. Jones, R. J. Dimler, Allene Jeanes, C. A. Wilham and C. E. Rist, *Abstr. Papers, Amer. Chem. Soc.*, **126**, 13 D (1954); a somewhat similar approach has been initiated independently in a study of the peripheral structure of limit dextrins from starch by D. French, J. Calamari and G. M. Wild, *ibid.* **122**, 5R (1952).

(4) (a) K. Freudenberg, W. Kuhn, W. Dürr, F. Bolz and G. Steinbrunn, *Ber.*, **63**, 1510 (1930); (b) K. Freudenberg and G. Blomqvist, *ibid.*, **68**, 2070 (1935).

(5) K. Freudenberg, *Trans. Faraday Soc.*, **32**, 74 (1936).

(6) L. G. Sillén, *Svensk Kem. Tidskr.*, **55**, 221 and 266 (1943).

(7) M. L. Wolfrom and J. C. Dacons, THIS JOURNAL, **74**, 5331 (1952).

(8) K. H. Meyer, H. Hopff and H. Mark, *Ber.*, **62**, 1103 (1929).

(9) K. Myrbäck and B. Magnusson, *Arkiv Kemi, Mineral. Geol.*, **A20**, No. 14, 22 pp. (1945).

(10) K. Myrbäck, B. Örtenblad and K. Ahlborg, *Biochem. Z.*, **307**, 53 (1940).

(11) B. Carlqvist, *Acta Chem. Scand.*, **2**, 759 (1948).

(12) K. Myrbäck, *Arkiv Kemi*, **1**, 161 (1949).

(13) Marjorie A. Swanson and C. F. Cori, *J. Biol. Chem.*, **172**, 797 (1948).

(14) Allene Jeanes, C. A. Wilham, R. W. Jones, H. M. Tsuchiya and C. E. Rist, THIS JOURNAL, **75**, 5911 (1953).

EXHIBIT 8
A132 – A136

CHEMURGIC DIGEST

May, 1958

CAN CHEMURGY SOLVE THE SURPLUS?

Dr. Walter H. C. Rueggeberg, in his thoughtful and provocative paper before the annual Conference, pointed out that the annual increase in carry-over of corn, about 150 million bushels, if entirely converted to starch would approximate the present total annual sales of starch. The corn now in government storage would keep the starch industry going for about four years!

This does not take into account the sorghums, wheat, and other starch-producing cereals, nor potatoes.

Thoughtful chemurgists have never asserted that chemurgy alone could "solve" the surplus problem; neither have they said that it could not do so. Their view has been that new uses and new crops certainly were a vitally important part of the solution; that a great new crop break-through might do it all; that the existing incentives to continue to produce surpluses are probably an impassable barrier; and that every economically practicable means to enlarge non-food utilization and to develop new industrial-use crops should be pursued vigorously. We should like to wipe out the whole surplus problem, but whatever chips we do take off are so much gains to agriculture, to taxpayers and the national economy. Everybody wins with every chemurgic advance, be it small or sweeping.

Because starch is the overwhelming and conspicuous phase of surplus, the Chemurgic Council takes satisfaction to be able in this issue to present the Conference papers on amylose: a *new* crop out of an *old* crop.

Industry needs amylose. Farmers know how to grow corn. Here is something you can call either a new use or a new crop. It cuts into the center of the corn surplus. May it prosper beyond all expectations!

THE OFFICIAL MAGAZINE OF

THE COUNCIL FOR AGRICULTURAL AND CHEMURGIC RESEARCH

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Research to Utilize Amylose, *by F. R. Senti* 7

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REMARKS FROM THE CHAIR

By Wheeler McMillen

Your Council can find more urgent jobs to work at than it has staff or funds to overtake. We are conscious, very conscious, that your Digest can be conspicuously improved. That's perhaps the uppermost job of all. Then, it should be possible to circulate it widely to educational institutions and similar places where memberships are not held, but where its content would inform or inspire. Even in its inadequate state the Digest appears to be highly regarded. The substantial number of bound volumes ordered each year attests to that.

From time to time important chemurgic material, too lengthy for the Digest, becomes available. We should like to print and circulate more things of that kind.

As an indication of widespread interest in chemurgic ideas and material, it has been urged that the Council undertake to arrange permanent or traveling exhibits for museums, and assurance has been given that such exhibits would receive good showings.

A staff executive could be of great service if he were on the move amongst industrial research leaders, government and state experiment stations, and farm groups. This kind of liaison could be highly stimulating to chemurgic progress.

The Special Program Evaluation Committee mentioned some of these ideas, and others. We shall try to find the resources to accomplish as many as possible.

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Figures indicate that for fiscal 1959 the USDA will have about $50 millions for production research projects, and about $26 millions for utilization research, including the $5 million to be done abroad and paid for with foreign currency received by the U. S. for surplus farm products.

The figure for production research is none too large. As Secretary Peterson told the Chemurgic Conference, we have to keep researching just to

The Story of Amylomaize Hybrids

By Robert P. Bear
Bear Hybrid Corn Co., Inc., Decatur, Illinois

CHEMURGY has been defined as the development of new, non-food uses for established farm crops, the adaptation of new crops to old uses, or the discovery of profitable uses for agricultural wastes. In addition to these definitions, I would like to add another: the development of a new crop for both old and new uses. In the development of amylomaize, we are designing, through breeding, a crop to supply a new industrial raw material for which the chemists believe there is a sizeable potential market.

"What is amylomaize?" Amylomaize is a generic term used to describe corn with amylose content above 50% which can be agronomically adapted to commercial production. The term "high amylose corn" is not particularly resonant. Some genetic oddities with high amylose are neither desirable nor practical for chemurgic development. Amylomaize, by definition, eliminates several high amylose genotypes, but it does describe those which have practical applications.

The development of amylomaize might be described as a reverse application of the chemurgic idea. Usually there is a surplus of an agricultural raw material and chemists are asked to find uses for it. With high-amylose starch, however, chemists have had some applications in mind for years, but have had no crop source that could supply a volume of raw material. So far as is known, there is no plant, except wrinkled peas, that naturally produces over 50% amylose starch.

Corn is grown in almost every country of the world, but so far, no corn with over 50% amylose has ever been found growing "wild." So amylomaize is a chemurgic crop developed through genetics. By increasing the amylose percentage of corn—thus providing an abundance of new raw material, industry will undoubtedly meet the challenge and find uses for this new raw product.

Presented before the 23rd National Farm Chemurgic Conference, Chicago, April 23, 1958.

Why We Started

To find out why amylomaize breeding was started, let's go back to the year 1948—ten years ago—when we started our research to see if corn could be genetically bred to produce high amylose starch. What was the picture at that time?

1. A market would be available for unknown millions of bushels of high amylose corn.
2. In this part of the world, corn is the cheapest source of starch in the plant kingdom.
3. Ordinary corn which had been grown for centuries had starch that analyzed approximately 25% amylose and 75% amylopectin.
4. Through mutations, corn hybrids of 100% amylopectin were being produced. Amylose had been eliminated entirely and mid-western farms were producing a chemurgic crop of hybrid corn containing 100% amylopectin starch.
5. Since we had bred good Tapicorn hybrids, containing only the amylopectin fraction of starch, it should be possible to breed a corn containing 100% of the other type of starch, amylose.
6. A book had been published a short time before—in 1946—entitled "New Riches from the Soil" by our good friend, Wheeler McMillen. While amylose starch was not mentioned there as a chemurgic possibility, the inspiration that I got from his writing fanned the ember of interest into a flame of action.

So I made a trip to the United States Department of Agriculture Regional Research Laboratory at Peoria, Illinois, to discuss the idea with Dr. Carl Rist, Dr. Fred Senti, Dr. M. M. MacMasters, Dr. G. E. Hilbert, and others. Their interest added impetus to the project and they agreed to run whatever chemical analyses we felt were necessary. So we proceeded to isolate and analyze many corn endosperm types that had occurred in our breeding nursery during the previous fifteen years.

Analyses Vital Aid

The development of amylomaize by Bear Hybrids has been a cooperative project with the USDA Peoria Laboratory. Without the chemical analyses and encouragement from the folks there during the last ten years, the amylomaize program would not be as far along as it is now. They are doing a monumental analysis job for us at Bear Hybrids as well as for the breeders at the University of Missouri under the direction of Dr. M. S. Zuber.

Robert P. Bear

It was during the routine analysis of breeding material in 1950 that we discovered a mutation in an inbred line that doubled the amylose in ordinary corn from 25% to 55%. Here was a single gene that accomplished as much in increasing amylose as had previously been accomplished by combinations of two or more genes. In addition, and more important from a practical standpoint, this gene did not decrease the total amount of starch produced and it did not increase the amount of water soluble polysaccharides. These last two items had eliminated all previous high amylose genotypes from any practical commercial consideration. This gene which we discovered has been named *ae*, for amylose extender.

The discovery of the *ae* gene fanned the interest in high amylose starch. While the amylose percent was below the desired level, it was a tremendous stride in a practical direction. Since this one gene made such a radical change, there must be others that would raise the amylose

level even higher. So far, no other gene has been found that increases amylose as much *ae* does. Our Research Director,, M. L. Vineyard, has, however, found several additional *ae* mutants in dent inbreds. I am confident that there will be other amylose increasing genes discovered in the future.

Sights Are Raised

In 1948, the chemists' desire was a 60% amylose starch—a rather fantastice figure since ordinary corn contained only 25%. During the years since then the processors have repeatedly raised their desired figure to 70—80—90%. To the plant breeders, it has seemed that the processors raised their desires just about the time we were actually coming up with higher percentages. We corn breeders believe that the chemists won't be really satisfied until we can supply them with corn in the neighborhood of 105% amylose.

A tremendous breeding job is necessary to take high amylose genetic material and convert it into practical inbred lines that can be used to make good hybrids, hybrids that farmers will grow by the thousands of acres and at a reasonable cost to industry for the raw material. The breeding requires a minimum of twelve generations to convert high amylose source stocks into practical hybrids that yield and perform as well as ordinary corn hybrids that are familiar to all midwest farmers. Only one generation of corn can be produced each summer in the midwest—each generation requires from 4 to 5 months to mature. We have been able to get twelve generations in six years by growing a second generation every year in our winter breeding nursery in the Rio Grande Valley of Texas.

Besides the time involved, the introduction of the *ae* gene into practical inbred lines presented no problems—at least so we thought. It was only a single gene transfer. We had used a similar procedure in transferring the single *wx* gene with no particular problems. (The *wx* gene is the one responsible for 100% amylopectin in starch.)

Gains in Know-How

After twelve generations, analyses were run on our amylose breeding material, and an alarming development was discovered. The amylose content on some lines was not as high as it should be—in fact in some cases it had dropped almost 10%.

One disadvantage inherent with being first to initiate such a breeding program is the tendency to predict results on the basis of previous experience. In this case the results were quite independent of expectations.

During the period 1950-1956, the number of amylose determinations made on all material was extremely limited. Certain assumptions were made to limit the analyses required, because to run each chemical analysis, from four to eight man hours were required.

After it was found that the original amylose percent of the *ae* gene could not be transferred automatically by conventional breeding methods, the number of amylose determinations was increased. Now we know how to maintain and even increase the amylose percentage during this period of transference from genetic material to practical inbred lines, but it requires extensive amylose analyses.

The detailed breeding methods are presented and discussed in two articles that have been submitted by us for publication to the *Agronomy Journal* and should appear in that publication late this year.

First Million Bushels?

Problems in the development of a new product or process are to be expected as a necessary evil. So a logical question is, "Just what is the present status of amylomaize hybrid development?" or "How soon will the first million bushels of amylomaize be produced?"

We have produced practical hybrids in the 50-60% amylose range—experimentally. The first million bushels of 50% amylomaize cannot be produced before 1961 and then only if industry has found uses which justify that production before the 1959 planting season—one year from now. How can I be so sure? The timetable is as inflexible as the laws of nature. In 1958, inbred lines that have already been developed will be increased in volume. In 1959, those inbred lines will be crossed to make single crosses. Then in 1960, the single crosses will be used to make double crosses—the seed that farmers can plant in 1961 to produce that first million bushels. *Please note* that I am not predicting that the first million bushels will be produced in 1961—only that it would be possible if the industrial uses warrant it within the next year.

What industrial research is being done on uses of this 50-60% amylose material? Dr. Fred Senti will cover much of this in his paper to this conference. In this summer of 1958, through the cooperation of American Maize Products Co. and the National Starch Products Co., the available supply of the first amylomaize hybrid seed ever produced will be grown for experimental processing, seed that was produced as a result of Bear Hybrid Corn Co. breeding during the last ten years. For the benefit of industries other than the wet milling industry, experimental amounts of this 50% amylomaize will be available at the end of the 1958 production year. There is no doubt that as various industries experiment with this new raw material many uses will be found for amylomaize.

The Higher Ranges

Now the next question is, "How about amylomaize in ranges above 60%?" With present knowledge, hybrids can be made in the 60-70% range. These hybrids will be acceptable to farmers from a performance standpoint. *Please note* that I said "can be made" when I talk about the 60-70% range. They are not yet in production—the inbred lines are still in the development stage.

Now let's speculate on the 70-80% range. In the near future (the near future for corn breeders means any time within ten years) it will be possible to make hybrids in that range without any additional basic knowledge. What we hope and expect to happen, however, is that increased amounts of basic research will uncover facts that will cut the time needed to get into that range—at least with the first inbred lines. At Bear Hybrids, we have had a considerable number of headings in the 70's from breeding material but there has not been sufficient time to transfer that amylose percentage to good inbred lines.

As far as the 80-100% amylose range is concerned, all I can say is that we are optimistic as we have always been about the possibilities for breeding high amylose corn. So far we have not been disappointed. But in order to make hybrids in the 80-100% range, additional knowledge will be needed—to use a popular term—a "break through" is needed.

This can only be accomplished by more basic research, the kind that we have been doing, the kind that is being done by Dr. Zuber at the University of Missouri, and the kind that is being done by Dr. Kramer at Purdue University. After 90% amylose is produced in genetic material, the transference of this percentage to good agronomic inbred lines should not be any more difficult than transference of 60% is now.

Dreams Become Realities

The chemurgic dream of ten years ago has become something of a reality today. During that period, the amylose content of ordinary corn was doubled almost overnight by the discovery of a new high amylose producing gene. In certain selections, this gene is producing breeding material containing three times as much amylose as is found in ordinary corn, with little or no loss in recoverable starch. This amylose producing gene has been successfully transferred to inbred lines which have been combined to produce practical amylomaize hybrids. Farmers will grow these hybrids in whatever volume the chemurgic applications justify. The possibilities of 90% amylomaize in the future are brighter today than the present level of 60% was ten years ago.

A vast new era is opening, an era which will bring further development in the chemurgic possibilities of corn through genetic breeding. Tapicorn hybrids are an established chemurgic crop in the United States today. The new amylomaize hybrids are the chemurgic corn crop of tomorrow. The developments to date are only a sample of genetic alterations that will be made in the chemical and physical properties of corn. The changes that have been accomplished in redesigning the starch fraction will, in the future, be duplicated in the oil, protein, vitamin, and other constituent parts of the corn kernel.

As breeders of corn specialties, we know that the chemurgic crop for the day after tomorrow will be one of several other corn varieties now in the laboratory stage of development. It is through such conferences as this that plant breeders can introduce the possibilities of brand new raw materials made possible by the application of genetics, imagination, and perspiration.

Research to Utilize Amylose

By F. R. Senti

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THE Northern Utilization Research and Development Division has been interested in the potential industrial utilization of amylose and high-amylose starches since the early 1940's and considerable research has been carried out in this field. Significant recent progress in developing high-amylose corn makes an appraisal of the current status and future outlook opportune at this time.

In this paper is presented a brief discussion of (a) the properties of amylose which distinguish it from normal starch and the amylopectin component of starch and their importance for the utilization of amylose and high-amylose starches, (b) the cooperative research program of the Northern Division on the development of high-amylose starches, (c) the present status of knowledge of properties of high-amylose starches, and (d) potential applications of high-amylose starch.

Most starches, including those from cereal grains, consist of two fractions called "amylose" and "amylopectin," present in the ratio of approximately 1 part amylose to 3 parts amylopectin. Both are polymers of the simple sugar glucose but they differ in that amylose molecules consist of glucose residues joined together to form long, linear chains whereas in amylopectin the molecules are branched at frequent intervals. This structural difference in the two starch components is depicted in Figure 1 and accounts to a large extent for the marked differences in their physical properties.

Amylose can be selectively precipitated from dilute starch solution by addition of butyl alcohol as was first demonstrated by T. J. Schoch (1) in 1941, or by any of a number of polar organic compounds, as Whistler and Hilbert (2) at the Northern Division showed, leaving amylopectin in solution. Selective precipitation with an organic liquid has not been developed into a commercial fractionation process. This method has been used extensively for laboratory preparation of relatively pure amylose

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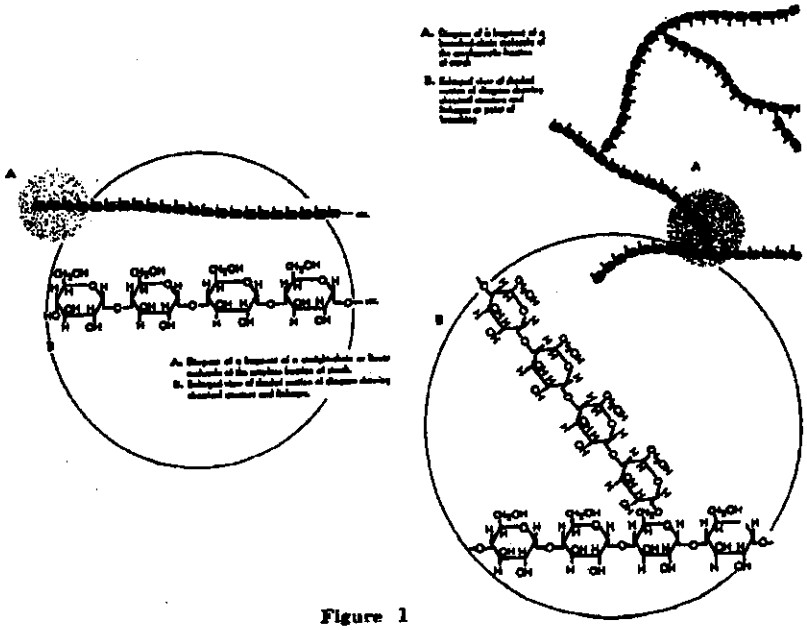

Figure 1